8. Procedure

8.1 Ambient receiving water must be used as the ''receiving water'' in the test. The temperature of the test water shall be as close as practicable to the ambient conditions in the receiving water, not the room temperature of the observation facility. The test container must have an air-to-liquid interface area of 1000 ±50 cm^2. The surface of the water should be no more than 1.27 cm (.5 inch) below the top of the test container.

8.2 Plastic liners shall be used, one per test container, and discarded afterwards. Some liners may inhibit spreading of added oil; operators shall determine an appropriate local source of liners that do not inhibit the spreading of the oil film.

8.3 A 15-mL sample of drilling fluid or well treatment, completion, and workover fluids must be introduced by pipette into the test container 1 cm below the water surface. Pipettes must be filled and discharged with test material prior to the transfer of test material and its introduction into test containers. The test water/test material mixture must be stirred using the pipette to distribute the test material homogeneously throughout the test water. The pipette must be used only once for a test and then discarded.

8.4 Drill cuttings or produced sand should be weighed on plastic weighing boats; 15-g samples must be transferred by scraping test material into the test water with a stainless steel spatula. Drill cuttings shall not be prediluted prior to testing. Also, drilling fluids and cuttings will be tested separately. The weighing boat must be immersed in the test water and scraped with the spatula to transfer any residual material to the test container. The drill cuttings or produced sand must be stirred with the spatula to an even distribution of solids on the bottom of the test container.

8.5 Observations must be made no later than 1 hour after the test material is transferred to the test container. Viewing points above the test container should be made from at least three sides of the test container, at viewing angles of approximately 60° and 30° from the horizontal. Illumination of the test container must be representative of adequate lighting for a working environment to conduct routine laboratory procedures. It is recommended that the water surface of the test container be observed under a fluorescent light source such as a dissecting microscope light. The light source shall be positioned above and directed over the entire surface of the pan.

8.6 Detection of a ''silvery'' or ''metallic'' sheen or gloss, increased reflectivity, visual color, iridescence, or an oil slick on the water surface of the test container surface shall constitute a demonstration of ''free oil.'' These visual observations include patches, streaks, or sheets of such altered surface characteristics. If the free oil content of the sample approaches or exceeds 10%, the water surface of the test container may lack color, a sheen, or iridescence, due to the increased thickness of the film; thus, the observation for an oil slick is required. The surface of the test container shall not be disturbed in any manner that reduces the size of any sheen or slick that may be present.

If an oil sheen or slick occurs on less than one-half of the surface area after the sample is introduced to the test container, observations will continue for up to 1 hour. If the sheen or slick increases in size and covers greater than one-half of the surface area of the test container during the observation period, the discharge of the material shall cease. If the sheen or slick does not increase in size to cover greater than one-half of the test container surface area after one hour of observation, discharge may continue and additional sampling is not required.

If a sheen or slick occurs on greater than one-half of the surface area of the test container after the test material is introduced, discharge of the tested material shall cease. The permittee may retest the material causing the sheen or slick. If subsequent tests do not result in a sheen or slick covering greater than one-half of the surface area of the test container, discharge may continue.

APPENDIX 2 TO SUBPART A OF PART 435—DRILLING FLUIDS TOXICITY TEST (EPA METHOD 1619)

I. Sample Collection

The collection and preservation methods for drilling fluids (muds) and water samples presented here are designed to minimize sample contamination and alteration of the physical or chemical properties of the samples due to freezing, air oxidation, or drying.

I-A. Apparatus

(1) The following items are required for water and drilling mud sampling and storage:

a. Acid-rinsed linear-polyethylene bottles or other appropriate noncontaminating drilling mud sampler.

b. Acid-rinsed linear-polyethylene bottles or other appropriate noncontaminating water sampler.

c. Acid-rinsed linear-polyethylene bottles or other appropriate noncontaminated vessels for water and mud samples.

d. Ice chests for preservation and shipping of mud and water samples.

I-B. Water Sampling

(1) Collection of water samples shall be made with appropriate acid-rinsed linear-polyethylene bottles or other appropriate

non-contaminating water sampling devices. Special care shall be taken to avoid the introduction of contaminants from the sampling devices and containers. Prior to use, the sampling devices and containers should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in 10 percent hydrochloric acid (HCl) for 4 hours, and then thoroughly rinsed with glass-distilled water.

I-C. Drilling Mud Sampling

(1) Drilling mud formulations to be tested shall be collected from active field systems. Obtain a well-mixed sample from beneath the shale shaker after the mud has passed through the screens. Samples shall be stored in polyethylene containers or in other appropriate uncontaminated vessels. Prior to sealing the sample containers on the platform, flush as much air out of the container by filling it with drilling fluid sample, leaving a one inch space at the top.

(2) Mud samples shall be immediately shipped to the testing facility on blue or wet ice (do not use dry ice) and continuously maintained at 0–4 °C until the time of testing.

(3) Bulk mud samples shall be thoroughly mixed in the laboratory using a 1000 rpm high shear mixer and then subdivided into individual, small wide-mouthed (e.g., one or two liter) non-contaminating containers for storage.

(4) The drilling muds stored in the laboratory shall have any excess air removed by flushing the storage containers with nitrogen under pressure anytime the containers are opened. Moreover, the sample in any container opened for testing must be thoroughly stirred using a 1000 rpm high shear mixer prior to use.

(5) Most drilling mud samples may be stored for periods of time longer than 2 weeks prior to toxicity testing provided that proper containers are used and proper condition are maintained.

II. Suspended Particulate Phase Sample Preparation

(1) Mud samples that have been stored under specified conditions in this protocol shall be prepared for tests within three months after collection. The SPP shall be prepared as detailed below.

II-A. Apparatus

(1) The following items are required:

a. Magnetic stir plates and bars.

b. Several graduated cylinders, ranging in volume from 10 mL to 1 L

c. Large (15 cm) powder funnels.

d. Several 2-liter graduated cylinders.

e. Several 2-liter large mouth graduated Erlenmeyer flasks.

(2) Prior to use, all glassware shall be thoroughly cleaned. Wash all glassware with detergent, rinse five times with tap water, rinse once with acetone, rinse several times with distilled or deionized water, place in a clean 10-percent (or stronger) HCl acid bath for a minimum of 4 hours, rinse five times with tap water, and then rinse five times with distilled or deionized water. For test samples containing mineral oil or diesel oil, glassware should be washed with petroleum ether to assure removal of all residual oil.

NOTE: If the glassware with nytex cups soaks in the acid solution longer than 24 hours, then an equally long deionized water soak should be performed.

II-B. Test Seawater Sample Preparation

(1) Diluent seawater and exposure seawater samples are prepared by filtration through a 1.0 micrometer filter prior to analysis.

(2) Artificial seawater may be used as long as the seawater has been prepared by standard methods or ASTM methods, has been properly ''seasoned,'' filtered, and has been diluted with distilled water to the same specified 20±2 ppt salinity and 20±2 °C temperature as the ''natural'' seawater.

II-C. Sample Preparation

(1) The pH of the mud shall be tested prior to its use. If the pH is less than 9, if black spots have appeared on the walls of the sample container, or if the mud sample has a foul odor, that sample shall be discarded. Subsample a manageable aliquot of mud from the well-mixed original sample. Mix the mud and filtered test seawater in a volumetric mud-to-water ratio of 1 to 9. This is best done by the method of volumetric displacement in a 2–L, large mouth, graduated Erlenmeyer flask. Place 1000 mL of seawater into the graduated Erlenmeyer flask. The mud subsample is then carefully added via a powder funnel to obtain a total volume of 1200 mL. (A 200 mL volume of the mud will now be in the flask).

The 2–L, large mouth, graduated Erlenmeyer flask is then filled to the 2000 mL mark with 800 mL of seawater, which produces a slurry with a final ratio of one volume drilling mud to nine volumes water. If the volume of SPP required for testing or analysis exceeds 1500 to 1600 mL, the initial volumes should be proportionately increased. Alternatively, several 2–L drill mud/water slurries may be prepared as outlined above and combined to provide sufficient SPP.

(2) Mix this mud/water slurry with magnetic stirrers for 5 minutes. Measure the pH and, if necessary, adjust (decrease) the pH of the slurry to within 0.2 units of the seawater by adding 6N HCl while stirring the slurry. Then, allow the slurry to settle for 1 hour. Record the amount of HCl added.

(3) At the end of the settling period, carefully decant (do not siphon) the Suspended Particulate Phase (SPP) into an appropriate container. Decanting the SPP is one continuous action. In some cases no clear interface will be present; that is, there will be no solid phase that has settled to the bottom. For those samples the entire SPP solution should be used when preparing test concentrations. However, in those cases when no clear interface is present, the sample must be remixed for five minutes. This insures the homogeneity of the mixture prior to the preparation of the test concentrations. In other cases, there will be samples with two or more phases, including a solid phase. For those samples, carefully and continuously decant the supernatant until the solid phase on the bottom of the flask is reached. The decanted solution is defined to be 100 percent SPP. Any other concentration of SPP refers to a percentage of SPP that is obtained by volumetrically mixing 100 percent SPP with seawater.

(4) SPP samples to be used in toxicity tests shall be mixed for 5 minutes and must not be preserved or stored.

(5) Measure the filterable and unfilterable residue of each SPP prepared for testing. Measure the dissolved oxygen (DO) and pH of the SPP. If the DO is less than 4.9 ppm, aerate the SPP to at least 4.9 ppm which is 65 percent of saturation. Maximum allowable aeration time is 5 minutes using a generic commercial air pump and air stone. Neutralize the pH of the SPP to a pH 7.8 ±.1 using a dilute HCl solution. If too much acid is added to lower the pH saturated NaOH may be used to raise the pH to 7.8 ±.1 units. Record the amount of acid or NaOH needed to lower/raise to the appropriate pH. Three repeated DO and pH measurements are needed to insure homogeneity and stability of the SPP. Preparation of test concentrations may begin after this step is complete.

(6) Add the appropriate volume of 100 percent SPP to the appropriate volume of seawater to obtain the desired SPP concentration. The control is seawater only. Mix all concentrations and the control for 5 minutes by using magnetic stirrers. Then, the animals shall be randomly selected and placed in the dishes in order to begin the 96-hour toxicity test.

III. Guidance for Performing Suspended Particulate Phase Toxicity Tests Using Mysidopsis bahia

III-A. Apparatus

(1) Each definitive test consists of 18 test containers: 3 replicates of a control and 5 SPP dilutions. Test containers should be Pyrex or equivalent glass. For definitive tests, 5 SPP dilutions with 3 replicates of at least 500 ml each are required. Twenty mysids per replicate, 360 per definitive test are required.

III-B. Sample Collection Preservation

(1) Drilling muds and water samples are collected and stored, and the suspended particulate phase prepared as described in section 1–C.

III-C. Species Selection

(1) The Suspended Particulate Phase (SPP) tests on drilling muds shall utilize the test species *Mysidopsis bahia*. Test animals shall be 3 to 6 days old on the first day of exposure. Whatever the source of the animals, collection and handling should be as gentle as possible. Transportation to the laboratory should be in well-aerated water from the animal culture site at the temperature and salinity from which they were cultured. Methods for handling, acclimating, and sizing bioassay organisms given by Borthwick [1] and Nimmo [2] shall be followed in matters for which no guidance is given here.

III-D. Experimental Conditions

(1) Suspended particulate phase (SPP) tests should be conducted at a salinity of 20 ±2 ppt. Experimental temperature should be 20 ±2 °C. Dissolved oxygen in the SPP shall be raised to or maintained above 65 percent of saturation prior to preparation of the test concentrations. Under these conditions of temperature and salinity, 65 percent saturation is a DO of 5.3 ppm. Beginning at Day 0-before the animals are placed in the test containers DO, temperature, salinity, and pH shall be measured every 24 hours. DO should be reported in milligrams per liter.

(2) Aeration of test media is required during the entire test with a rate estimated to be 50–140 cubic centimeters/minute. This air flow to each test dish may be achieved through polyethylene tubing (0.045-inch inner diameter and 0.062-inch outer diameter) by a small generic aquarium pump. The delivery method, surface area of the aeration stone, and flow characteristics shall be documented. All treatments, including control, shall be the same.

(3) Light intensity shall be 1200 microwatts/cm^2 using cool white fluorescent bulbs with a 14-hr light and 10-hr dark cycle. This light/dark cycle shall also be maintained during the acclimation period and the test.

III-E. Experimental Procedure

(1) Wash all glassware with detergent, rinse five times with tap water, rinse once with acetone, rinse several times with distilled or deionized water, place in a clean 10 percent HCl acid bath for a minimum of 4 hours, rinse five times with tap water, and then rinse five times with distilled water.

(2) Establish the definitive test concentrations based on results of a range finding test or based on prior experience and knowledge of the mud system.

(3) Twenty organisms are exposed in each test dish. Nytex® cups shall be inserted into every test dish prior to adding the animals. These ''nylon mesh screen'' nytex holding cups are fabricated by gluing a collar of 363-micrometer mesh nylon screen to a 15-centimeter wide Petri dish with silicone sealant. The nylon screen collar is approximately 5 centimeters high. The animals are then placed into the test concentration within the confines of the Nytex cups.

(4) Individual organisms shall be randomly assigned to treatment. A randomization procedure is presented in section V of this protocol. Make every attempt to expose animals of approximately equal size. The technique described by Borthwick [1], or other suitable substitutes, should be used for transferring specimens. Throughout the test period, mysids shall be fed daily with approximately 50 *Artemia* (brine shrimp) nauplii per mysid. This will reduce stress and decrease cannibalism.

(5) Cover the dishes, aerate, and incubate the test containers in an appropriate test chamber. Positioning of the test containers holding various concentrations of test solution should be randomized if incubator arrangement indicates potential position difference. The test medium is not replaced during the 96-hour test.

(6) Observations may be attempted at 4, 6 and 8 hours; they must be attempted at 0, 24, 48, and 72 hours and must be made at 96 hours. Attempts at observations refers to placing a test dish on a light table and visually counting the animals. Do not lift the ''nylon mesh screen'' cup out of the test dish to make the observation. No unnecessary handling of the animals should occur during the 96 hour test period. DO and pH measurements must also be made at 0, 24, 48, 72, and 96 hours. Take and replace the test medium necessary for the DO and pH measurements outside of the nytex cups to minimize stresses on the animals.

(7) At the end of 96 hours, all live animals must be counted. Death is the end point, so the number of living organisms is recorded. Death is determined by lack of spontaneous movement. All crustaceans molt at regular intervals, shedding a complete exoskeleton. Care should be taken not to count an exoskeleton. Dead animals might decompose or be eaten between observations. Therefore, always count living, not dead animals. If daily observations are made, remove dead organisms and molted exoskeletons with a pipette or forceps. Care must be taken not to disturb living organisms and to minimize the amount of liquid withdrawn.

IV. Methods for Positive Control Tests (Reference Toxicant)

(1) Sodium lauryl sulfate (dodecyl sodium sulfate) is used as a reference toxicant for the positive control. The chemical used should be approximately 95 percent pure. The source, lot number, and percent purity shall be reported.

(2) Test methods are those used for the drilling fluid tests, except that the test material was prepared by weighing one gram sodium lauryl sulfate on an analytical balance, adding the chemical to a 100-milliliter volumetric flask, and bringing the flask to volume with deionized water. After mixing this stock solution, the test mixtures are prepared by adding 0.1 milliliter of the stock solution for each part per million desired to one liter of seawater.

(3) The mixtures are stirred briefly, water quality is measured, animals are added to holding cups, and the test begins. Incubation and monitoring procedures are the same as those for the drilling fluids.

V. Randomization Procedure

V-A. Purpose and Procedure

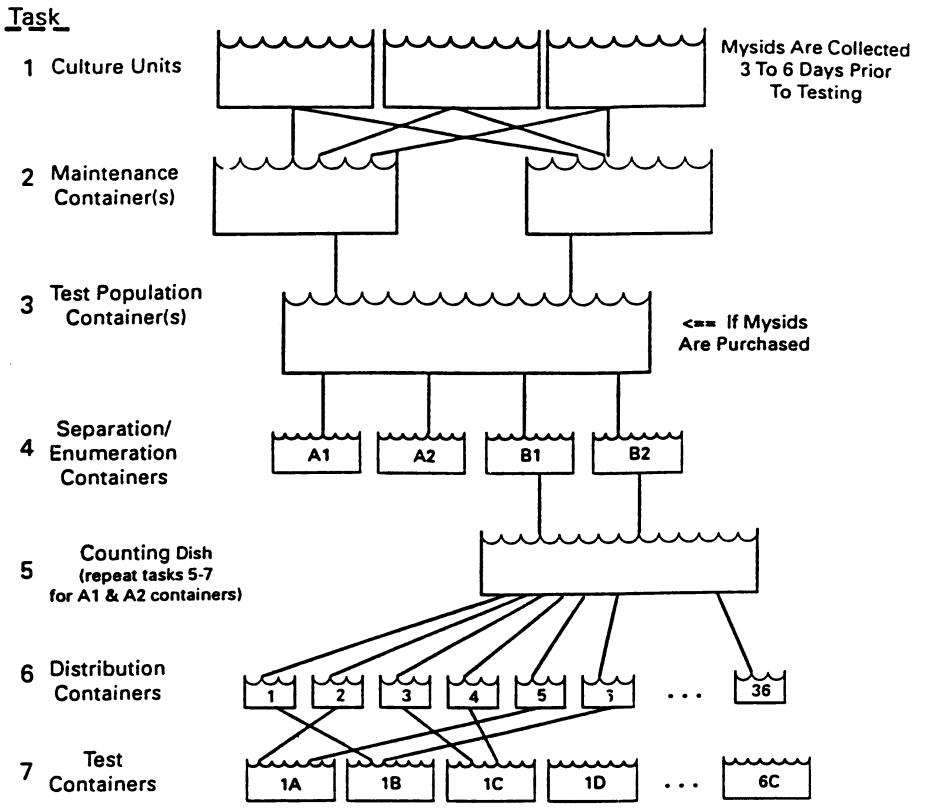
(1) The purpose of this procedure is to assure that mysids are impartially selected and randomly assigned to six test treatments (five drilling fluid or reference toxicant concentrations and a control) and impartially counted at the end of the 96-hour test. Thus, each test setup, as specified in the randomization procedure, consists of 3 replicates of 20 animals for each of the six treatments, *i.e.*, 360 animals per test. Figure 1 is a flow diagram that depicts the procedure schematically and should be reviewed to understand the over-all operation. The following tasks shall be performed in the order listed.

(2) Mysids are cultured in the laboratory in appropriate units. If mysids are purchased, go to Task 3.

(3) Remove mysids from culture tanks (6, 5, 4, and 3 days before the test will begin, *i.e.*, Tuesday, Wednesday, Thursday, and Friday if the test will begin on Monday) and place them in suitably large maintenance containers so that they can swim about freely and be fed.

NOTE: Not every detail (the definition of suitably large containers, for example) is provided here. Training and experience in aquatic animal culture and testing will be required to successfully complete these tests.

Figure 1
Mysid Randomization Procedure



(4) Remove mysids from maintenance containers and place all animals in a single container. The intent is to have homogeneous test population of mysids of a known age (3–6 days old).

(5) For each toxicity test, assign two suitable containers (500-milliliter (mL) beakers are recommended) for mysid separation/enumeration. Label each container (A1, A2, B1, B2, and C1, C2, for example, if two drilling fluid tests and a reference toxicant test are to be set up on one day). The purpose of this task is to allow the investigator to obtain a close estimate of the number of animals available for testing and to prevent unnecessary crowding of the mysids while they are being counted and assigned to test containers. Transfer the mysids from the large test population container to the labeled separation and enumeration containers but do not place more than 200 mysids in a 500-mL beaker. Be impartial in transferring the mysids; place approximately equal numbers of animals (10–15 mysids is convenient) in each container in a cyclic manner rather than placing the maximum number each container at one time.

NOTE: It is important that the animals not be unduly stressed during this selection and assignment procedure. Therefore, it will probably be necessary to place all animals (except the batch immediately being assigned to test containers) in mesh cups with flowing seawater or in large volume containers with aeration. The idea is to provide the animals with near optimal conditions to avoid additional stress.

(6) Place the mysids from the two labeled enumeration containers assigned to a specific test into one or more suitable containers to be used as counting dishes (2-liter Carolina dishes are suggested). Because of the time required to separate, count, and assign mysids, two or more people may be involved in completing this task. If this is done, two or more counting dishes may be used, but the investigator must make sure that approximately equal numbers of mysids from each labeled container are placed in each counting dish.

(7) By using a large-bore, smooth-tip glass pipette, select mysids from the counting dish(es) and place them in the 36 individually numbered distribution containers (10-ml beakers are suggested). The mysids are assigned two at a time to the 36 containers by using a randomization schedule similar to the one presented below. At the end of selection/assignment round 1, each container will contain two mysids; at the end of round 2, they will contain four mysids; and so on until each contains ten mysids.

EXAMPLE OF A RANDOMIZATION SCHEDULE

Selection/assignment round (2 mysids each)	Place mysid in the numbered distribution containers in the random order shown
1	8, 21, 6, 28, 33, 32, 1, 3, 10, 9, 4, 14, 23, 2, 34, 22, 36, 27, 5, 30, 35, 24, 12, 25, 11, 17, 19, 26, 31, 7, 20, 15, 18, 13, 16, 29.
2	35, 18, 5, 12, 32, 34, 22, 3, 9, 16, 26, 13, 20, 28, 6, 21, 24, 30, 8, 31, 7, 23, 2, 15, 25, 17, 1, 11, 27, 4, 19, 36, 10, 33, 14, 29.
3	7, 19, 14, 11, 34, 21, 25, 27, 17, 18, 6, 16, 29, 2, 32, 10, 4, 20, 3, 9, 1, 5, 28, 24, 31, 15, 22, 13, 33, 26, 36, 12, 8, 30, 35, 23.
4	30, 2, 18, 5, 8, 27, 10, 25, 4, 20, 26, 15, 31, 36, 35, 23, 11, 29, 16, 17, 28, 1, 33, 14, 9, 34, 7, 3, 12, 22, 21, 6, 19, 24, 32, 13.
5	34, 28, 16, 17, 10, 12, 1, 36, 20, 18, 15, 22, 2, 4, 19, 23, 27, 29, 25, 21, 30, 3, 9, 33, 32, 6, 14, 11, 35, 24, 26, 7, 31, 5, 13, 8.

(8) Transfer mysids from the 36 distribution containers to 18 labeled test containers in random order. A label is assigned to each of the three replicates (A, B, C) of the six test concentrations. Count and record the 96 hour response in an impartial order.

(9) Repeat tasks 5–7 for each toxicity test. A new random schedule should be followed in Tasks 6 and 7 for each test.

NOTE: If a partial toxicity test is conducted, the procedures described above are appropriate and should be used to prepare the single test concentration and control, along with the reference toxicant test.

V-B. Data Analysis and Interpretation

(1) Complete survival data in all test containers at each observation time shall be presented in tabular form. If greater than 10 percent mortality occurs in the controls, all data shall be discarded and the experiment repeated. Unacceptably high control mortality indicates the presence of important stresses on the organisms other than the material being tested, such as injury or disease, stressful physical or chemical conditions in the containers, or improper handling, acclimation, or feeding. If 10 percent mortality or less occurs in the controls, the data may be evaluated and reported.

(2) A definitive, full bioassay conducted according to the EPA protocol is used to estimate the concentration that is lethal to 50 percent of the test organisms that do not die naturally. This toxicity measure is known as the median lethal concentration, or LC–50. The LC–50 is adjusted for natural mortality or natural responsiveness. The maximum likelihood estimation procedure with the adjustments for natural responsiveness as given by D.J. Finney, in *Probit Analysis* 3rd edition, 1971, Cambridge University Press, chapter 7, can be used to obtain the probit model estimate of the LC–50 and the 95 percent fiducial (confidence) limits for the LC–50. These estimates are obtained using the logarithmic transform of the concentration. The heterogeneity factor (Finney 1971, pages 70–72) is not used. For a test material to pass the toxicity test, according to the requirements stated in the offshore oil and gas extraction industry BAT effluent limitations and NSPS, the LC–50, adjusted for natural responsiveness, must be greater than 3 percent suspended particulate phase (SPP) concentration by volume unadjusted for the 1 to 9 dilution. Other toxicity test models may be used to obtain toxicity estimates provided the modeled mathematical expression for the lethality rate must increase continuously with concentration. The lethality rate is modeled to increase with concentration to reflect an assumed increase in toxicity with concentration even though the observed lethality may not increase uniformly because of the unpredictable animal response fluctuations.

(3) The range finding test is used to establish a reasonable set of test concentrations in order to run the definitive test. However, if the lethality rate changes rapidly over a narrow range of concentrations, the range finding assay may be too coarse to establish

an adequate set of test concentrations for a definitive test.

(4) The EPA Environmental Research Laboratory in Gulf Breeze, Florida prepared a Research and Development Report entitled Acute Toxicity of Eight Drilling Fluids to Mysid Shrimp (*Mysidopsis bahia*), May 1984 EPA–600/3–84–067. The Gulf Breeze data for drilling fluid number 1 are displayed in Table 1 for purposes of an example of the probit analysis described above. The SAS Probit Procedure (SAS Institute, Statistical Analysis System, Cary, North Carolina, 1982) was used to analyze these data. The 96-hour LC50 adjusted for the estimated spontaneous mortality rate is 3.3 percent SPP with 95 percent limits of 3.0 and 3.5 percent SPP with the 1 to 9 dilution. The estimated spontaneous mortality rate based on all of the data is 9.6 percent.

TABLE 1—LISTING OF ACUTE TOXICITY TEST DATA (AUGUST 1983 TO SEPTEMBER 1983) WITH EIGHT GENERIC DRILLING FLUIDS AND MYSID SHRIMP

[fluid N2=1]

Percent concentration	Number exposed	Number dead (96 hours)	Number alive (96 hours)
0	60	3	57
1	60	11	49
2	60	11	49
3	60	25	35
4	60	48	12
5	60	60	0

V-C. The Partial Toxicity Test for Evaluation of Test Material

(1) A partial test conducted according to EPA protocol can be used economically to demonstrate that a test material passes the toxicity test. The partial test cannot be used to estimate the LC–50 adjusted for natural response.

(2) To conduct a partial test follow the test protocol for preparation of the test material and organisms. Prepare the control (zero concentration), one test concentration (3 percent suspended particulate phase) and the reference toxicant according to the methods of the full test. A range finding test is not used for the partial test.

(3) Sixty test organisms are used for each test concentration. Find the number of test organisms killed in the control (zero percent SPP) concentration in the column labeled X_0 of Table 2. If the number of organisms in the control (zero percent SPP) exceeds the table values, then the test is unacceptable and must be repeated. If the number of organisms killed in the 3 percent test concentration is less than or equal to corresponding number in the column labeled X_1 then the test material passes the partial toxicity test. Otherwise the test material fails the toxicity test.

(4) Data shall be reported as percent suspended particulate phase.

TABLE 2

X_0	X_1
0	22
1	22
2	23
3	23
4	24
5	24
6	25

VI. References

1. Borthwick, Patrick W. 1978. Methods for acute static toxicity tests with mysid shrimp (*Mysidopsis bahia*). Bioassay Procedures for the Ocean Disposal Permit Program, [EPA–600/9–78–010:] March.

2. Nimmo, D.R., T.L. Hamaker, and C.A. Somers. 1978. Culturing the mysid (*Mysidopsis bahia*) in flowing seawater or a static system. Bioassay Procedures for the Ocean Disposal Permit Program, [EPA–600/9–78–010]: March.

3. American Public Health Association et al. 1980. Standard Methods for the Examination of Water and Wastewater. Washington, DC, 15th Edition: 90–99.

4. U.S. Environmental Protection Agency, September 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA/600/4–90/027. Washington, DC, 4th Edition.

5. Finney, D.J. Probit Analysis. Cambridge University Press; 1971.

6. U.S. Environmental Protection Agency, May 1984. Acute Toxicity of Eight Drilling Fluids to Mysid Shrimp (*Mysidopsis bahia*). EPA–600/3–84–067.

[58 FR 12504, Mar. 4, 1993, as amended at 77 FR 29837, May 18, 2012]

APPENDIX 3 TO SUBPART A OF PART 435—PROCEDURE FOR MIXING BASE FLUIDS WITH SEDIMENTS (EPA METHOD 1646)

This procedure describes a method for amending uncontaminated and nontoxic (control) sediments with the base fluids that are used to formulate synthetic-based drilling fluids and other non-aqueous drilling fluids. Initially, control sediments shall be press-sieved through a 2000 micron mesh sieve to remove large debris. Then press-sieve the sediment through a 500 micron sieve to remove indigenous organisms that may prey on the test species or otherwise confound test results. Homogenize control sediment to limit the effects of settling that